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14. ABSTRACT The long-term goal of this training program is to increase veterinary research manpower by providing research training to veterinary students. This program has been training 83, 21, 26 and 10 DVM students in summer research, DVM/MS-LAM, DVM/MPH and DVM/MS-CBS programs, respectively since 2007 with 55 students still at TCSVM. The graduates of these programs are serving in US Army Veterinary Corps (4), enrolled in LAM residency program (6), conducting biomedical research (9), received Fogarty Clinical fellowship (2), enrolled in PhD program (5) or joined government agencies (2). The trainees published 5 peer-reviewed manuscripts and presented 22 abstracts in national and international scientific meetings. The goal of the program is considered to have been achieved as evidenced by the number of trainees recruited in the program and the activities of the graduates.					
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Table of Contents

	<u>Page</u>
Introduction.....	4
Body.....	4
Key Research Accomplishments.....	9
Reportable Outcomes.....	10
Conclusion.....	11
References.....	11
Appendix 1. DVM/MS-CBS thesis summary	13

INTRODUCTION:

The long-term goal of this training program is to increase veterinary research manpower by providing research training to veterinary students. The program is expected to develop a pipeline of research ready veterinarians to solve biodefense and public health problems facing our country, in general, and our armed forces in particular. The following training programs offered by Tufts Cummings School of Veterinary Medicine (TCSVM) were proposed for this purpose.

1. Summer Research Program
2. Four-year joint DVM/Master's degree in Public Health degree (DVM/MPH) program
3. Four-year joint DVM/Master of Science degree in Laboratory Animal Medicine (DVM/MS-LAM)
4. Five-year joint DVM/MS in Comparative Biomedical Science (DVM/MS-CBS)

During the summer research program, veterinary students are trained to conduct mentored research under the guidance of a faculty. Veterinary students are also recruited in joint degree programs that allow more specialized training in public health and laboratory animal medicine and more in-depth research training in comparative biomedical sciences.

Body:

This is a training grant with training opportunities in multiple programs, and this final progress report includes accomplishment from August, 2006 (Award date) to August 2013 of this multi-year program; the trainee recruitment started in 2007.

Program Implementation: As proposed in the application, veterinary students were informed of the training program soon after receiving the award notice. A website as well as a brochure was created to inform veterinary students and faculty (<http://www.tufts.edu/vet/researchtraining/defense.html>). The website included the program description, responsibilities of trainees and mentors, list of program faculty and individual program directors and an application package. In addition, students and faculty were informed of the program via email with reference to the website and a seminar was held to explain the program to students and program faculty. Prospective trainees were asked to contact program faculty for summer research projects and program directors for the joint-degree programs.

Recruitment: The goal of the program was to recruit trainees in the Summer Research Program, DVM-MS-CBS programs, DVM/MPH and DVM/MS-LAM. The trainees were selected based on the scientific merit of the proposal, academic standing in the veterinary school, relevance to biodefense and stated interest in an army career. The program recruited a total of 83, 21, 26 (2 left before completing) and 10 DVM students in the Summer Research Program, DVM/MPH, DVM/MS-LAM, and DVM-MS-CBS programs, respectively. All recruited trainees completed the assigned programs.

Progress: The following is the summary of progress for each program supported by this grant since 2007 (see table 1 for further details):

DVM/MS-LAM program: Twenty one trainees completed the MS-LAM portion this dual degree program since 2007; six are now completing the DVM portion of the program. Of the 15 graduates of this program 7 received or are receiving further training in LAM at various universities, 2 entered private practice, 2 are serving in US Army Vet. Corp, 2 are engaged in biomedical research and 2 are completing a PhD degree.

DVM/MPH program: Twenty six trainees participated in this program since 2007 and two left the program before completion. Twelve of the remaining 24 trainees graduated and the rest are still enrolled in the program. Of the 12 graduates 9 entered private practices, 1 serving US Army Vet. Corp., 1 joined USDA and 1 is an epidemiologist at Boston Public Health Commission.

DVM/MS-CBS program: Ten trainees participated in this program since 2007. Six of these trainees have graduated, 4 have submitted and defended their MS thesis and currently completing the DVM portion of the combined degree program. Of the 6 graduates, one is serving in US Army Veterinary Corps, one is participating in a post-doctoral fellowship program, one is completing a PhD degree and three are in private practice. The abstracts of the completed thesis are included in Appendix 1.

Summer Research Program: A total of 83 trainees participated in this program since 2007. Forty eight of these trainees have graduated and 35 trainees are still TCSVM student. Of the 48 graduates, 34 entered private practice, 5 are involved in research, 2 joined government agencies, one is serving in the US Army and 6 are involved in unknown activities (see table 1). All trainees submitted a written report and presented their research work at the annual veterinary student research day. They competed for three awards based on the written report and the oral presentation, and received the award at the end of the annual veterinary student research day. Note that all trainees in DVM/MS-CBS completed the summer research program before entering the combined degree program and 11 of them are also enrolled in the DVM/MPH program. Each trainee in the Summer Research Program is required to complete a survey to provide feedback on their research experience and future plan. The survey data from 83 trainees revealed the following: 90% had a stimulating research experience and 68% planned to obtain more research experience while in veterinary school, 24% considered entering a combined degree program, 81% were interested in getting involved in research after completing the DVM program and 8% were interested in joining the army.

All trainees were required to attend and attended a special information session by invited US army officers on career opportunities in the US Armed forces for veterinarians. These sessions were organized by the local US Army recruiting officers.

Table 1: List of trainee in various training programs (2007-2013)

A. DVM/MS-LAM Program

Name of Trainee	Training Program	Training Completion Year	DVM Graduation Year	Current position
Misty Williams	DVM/MS-LAM	2008	2008	Lab Animal Vet at CBSET, Lexington, MA
Kristina Asselin	DVM/MS-LAM	2010	2010	Clinical Vet at Princeton University
Elizabeth Carbone	DVM/MS-LAM	2013	2013	Resident, LAM, UC Davis
Jessica Connolly	DVM/MS-LAM	2009	2009	Serving US Army Vet. Corp.
Julia Goldman	DVM/MS-LAM	2011	2011	Lab animal Residency, University of Illinois, Chicago
Amanda Graveline	DVM/MS-LAM	2011	2011	Biomedical Research, Wyss Institute, Boston
Marian Schenk	DVM/MS-LAM	2014	2014	TCSVM student
Amory Koch	DVM/MS-LAM	2011	2011	Serving US Army Vet. Corps
Wenjun Li	DVM/MS-LAM	2009	2009	Biomedical Research
Lindsey Miller	DVM/MS-LAM	2010	2010	Ocean State Veterinary Specialists - small animal medicine and surgery internship
Mayrav Moreshet	DVM/MS-LAM	2009	2009	Lab Animal Vet, Harvard Med School
Morgan Oexner	DVM/MS-LAM	2013	2013	Resident, LAM, Yale University
Diane Peters	DVM/MS-LAM	2015	2015	Currently pursuing the PhD part of the combined DVM/PhD program
Melissa Timm	DVM/MS-LAM	2010	2010	LAM Resident at Wake Forest University

Stephanie Woods	DVM/MS-LAM	2011	2011	PhD student at MIT, Cambridge, MA
Charles Byrd	DVM/MS-LAM	2014	2014	TCSVM student
Cecilia de Souza	DVM/MS-LAM	2014	2014	TCSVM student
Rebecca LaFleur	DVM/MS-LAM	2014	2014	TCSVM student
Rebecca Lossing	DVM/MS-LAM	2014	2014	TCSVM student
Christina DeAngelo	DVM/MS-LAM	2014	2014	TCSVM student
Katie Lee	DVM/MS-LAM	2013	2013	Intern, Small Animal Medicine, New England Animal Medical Center

B. DVM/MPH Program

Name of Trainee	Training Program	Training Completion Year	DVM Graduation Year	Current position
Karen Alroy	DVM/MPH	2012	2012	Fogarty Scholar, Intern at Friendship Hospital for Animals, DC
Colin Basler	DVM/MPH	2012	2012	EIS Training Program
Lauren Bifano	DVM/MPH	2012	2012	Intern at Ocean State Veterinary Specialists, RI
Madalyn Black	DVM-MPH	2014	2014	TCSVM student
Stephen Diaz	DVM-MPH	2014	2014	TCSVM student
Anne Fleming	DVM/MPH	2013	2013	Veterinarian Associate at Spinnaker Veterinary Clinic
Kylynn Fontaine	DVM-MPH	2014	2014	TCSVM student
Elliott Garber	DVM/MPH	2009	2009	Serving US Army Vet. Corp.
Tammy Han	DVM/MPH	2009	2009	Private practice
Ashley-Rose Hart	DVM-MPH	2014	2014	TCSVM student
Miranda Hillyard	DVM/MPH	2011	2011	Intern at Bergh Memorial Animal Hospital, NY
Lauren Krone	DVM/MPH	2013	2013	Intern, Tufts University, Foster Hospital for Small Animals
Jennifer McRobbie	DVM-MPH	2010	2010	Epidemiologist at Boston Public Health Commission
Sarah Merriday	DVM-MPH	2014	2014	TCSVM student, Joined US Army Vet Corp
Cecilia Murch	DVM/MPH	2013	2013	Animal Hospital of Pittsford
Misha Robyn	DVM/MPH	2009	2009	USDA Public Health Veterinarian
Marieke Rosenbaum	DVM-MPH	2014	2014	TCSVM student
Emily Roye	DVM-MPH	2013	2013	Intern, Vetcision, MA
Sara Dale	DVM-MPH	2015	2015	TCSVM student
Philip "PJ" Hamel	DVM-MPH	2015	2015	TCSVM student
Shira Kedmi (Rothstein)	DVM-MPH	2015	2015	TCSVM student
Aliza Gentili-Lloyd	DVM-MPH	2015	2015	TCSVM student
Evin Luehrs	DVM-MPH	2015	2015	TCSVM student
Kyle Ross	DVM-MPH	2015	2015	TCSVM student

C. DVM/MS-CBS Program

Name of Trainee	Training Program	Training Completion Year	DVM Graduation Year	Current position
Elliott Garber	DVM/MS-CBS	2008	2009	Serving in US Army Vet. Corp.
Jessica Hekman	DVM/MS-CBS	2010	2012	PhD Student at Cornell
Katherine Meguire	DVM/MS-CBS	2010	2012	Intern in small animal hospital, Denver, CO

Marieke Rosenbaum	DVM/MS-CBS	2009	2014	TCSVM student
Karyn Vonlderstein	DVM/MS-CBS	2008	2010	Post-doctoral fellow, MGH, Boston
Jana Thomas	DVM/MS-CBS	2011	2013	Associate vet at Putnam Veterinary Clinic in Topsfield, MA
Bronwen Childs	DVM/MS-CBS	2012	2013	Intern, Peninsula Equine Medical Center
Marian Schenk	DVM/MS-CBS	2012	2014	Thesis Accepted
Cristina Carballo	DVM/MS-CBS	2012	2014	Thesis Accepted
Kristy Meadows	DVM/MS-CBS	2013	2015	Thesis Accepted

D. Summer Research Program

Name of Trainee	Training Completion year	DVM Graduation Year	Current Position
Jennifer McRobbie	2007	2010	Infectious Disease Epidemiologist – Boston Public Health Commission
Victoria Fields	2007	2010	Veterinarian – Veterinary Referral Center of Colorado
Miranda Hillyard	2007	2011	Intern/Small Animal - Bergh Memorial Animal Hospital of the ASPCA
Shannabeth Minior	2007	2009	MSPCA
Misha Park Robyn	2007	2009	USDA
Rebecca Steers	2007	2010	US Army
Tierra Wilson	2007	2010	PhD program at UC Davis' Wildlife Health Center
Karen Alroy	2008	2012	Intern/Small Animal – Friendship Hospital for Animals
Sarah Carter	2008	2010	Veterinarian - MSPCA at Nevins Farm
Philip Gerwin	2008	2011	Study Director, Toxicon Corporation, Bedford, MA
Sara Heslop	2008	2011	Intern - Oradell Animal Hospital
Miranda Hillyard	2008	2010	Intern/Small Animal - Bergh Memorial Animal Hospital of the ASPCA
Lauren O'Connell	2008	2011	Animal Medical Hospital of State College
Kathleen Riley	2008	2010	Staff Veterinarian - North Shore Animal League
Annie Shea	2008	2011	Intern - Angell Animal Medical Center
Angela Snell	2008	2011	Unknown
Christie Taylor	2008	2011	Associate Veterinarian –Banfield Pet Hospital
Stephen Wilson	2008	2011	Veterinarian - Arlington Animal Clinic, Inc.
Tierra Wilson/Smiley	2008	2010	PhD program at UC Davis' Wildlife Health Center
Colin Basler	2009	2012	Private practice
Ashley Case	2009	2012	Intern – Colorado State
Rebecca Foelber	2009	2012	Intern/Small Animal Intern – VCA Animal Hospitals
Matthew Gordon	2009	2012	Intern – BluePearl, NY
Katherine Haman	2009	2012	Unknown, left TCSVM in 2009
Susan Hayhurst	2009	2011	unknown
Jamie Lovejoy	2009	2012	Unknown
Lauren O'Connell	2009	2011	Animal Medical Hospital of State College
Sarah Raabis	2009	2012	Intern/Livestock Med. & Surgical - Colorado State University
Lydia Scheidler	2009	2011	Associate Veterinarian - South Bay Veterinary Group
Samantha Swisher	2009	2012	Associate Veterinarian - VCA Veterinary Referral

			Associates
Hannah Tadros	2009	2012	Unknown
Deborah Thomson	2009	2012	Intern – Veterinary Medical & Surgical Group
Alison Allukian	2010	2012	Intern – MSPCA, Angell Animal Medical Center
Bronwen Childs	2010	2013	Intern, Peninsula Equine Medical Center
Andreas Eleftheriou	2010	2013	Intern, University of Pennsylvania
Mariah Foose	2010	2013	Unknown
Laura Harvey	2010	2012	Intern – University of Georgia
Thanhthao Huynh	2010	2012	Small Animal Med. & Surgical Intern - 5th Avenue Veterinary Specialists
Jonathan Kuo	2010	2013	Veterinarian, VCA Alpine Animal Hospital
Heather McFarland	2010	2013	Associate Veterinarian, At Home Veterinary
Deborah Thomson	2010	2012	Intern – Veterinary Medical & Surgical Group
Burns, Monika	2011	2013	Postdoctoral Associate at Massachusetts Institute of Technology (MIT)
Chalam, Yamini	2011	2014	TCSVM Student
Fleming, Anne	2011	2013	Veterinarian Associate at Spinnaker Veterinary Clinic
Gordon, Max	2011	2014	TCSVM Student
Hamilton, Jessie	2011	2014	TCSVM Student
Holmes, Katherine	2011	2013	Rotating Intern Veterinarian, New England Animal Medical Center
Jacobus, Kristy	2011	2014	TCSVM Student
Khodari, Joe	2011	2013	Graduated from Univ. Pennsylvania
Lim, Sarah	2011	2014	TCSVM Student
Murch, Cecilia	2011	2013	Animal Hospital of Pittsford
Penrod, Casey	2011	2014	TCSVM Student
Pogue, Natasha	2011	2014	TCSVM Student
Roye, Emily	2011	2013	Intern, Vetcision
Sowy, Stanley	2011	2014	Student at Western University
Tucker, Casey	2011	2013	Associate Veterinarian, Holden Veterinary Clinic
Turner, Laura	2011	2014	TCSVM Student
Whitford, Annie	2011	2014	Student at LSU Veterinary School
Ash, Lesley	2012	2014	TCSVM student
Barnett, Grace	2012	2015	TCSVM student
Chalam, Yamini	2012	2014	TCSVM student
Chevett, Kelly	2012	2015	Student at Atlantic Veterinary College, University of Prince Edward Island
Connolly, Kevin	2012	2015	TCSVM student
Dale, Sarah	2012	2015	TCSVM student
Dickey, Meranda	2012	2015	TCSVM student
DiMeglio, Julie	2012	2014	TCSVM student
Glass, Melanie	2012	2015	TCSVM student
Hamilton, Jessie	2012	2014	TCSVM student
Jacobs, Rachel	2012	2014	TCSVM student
Jacobus, Kristy	2012	2014	TCSVM student
Kozol, Stephanie	2012	2015	TCSVM student
Morlock, Jillian	2012	2014	TCSVM student
Merriday, Sarah	2012	2014	TCSVM student
Moynehan, Shannon	2012	2015	TCSVM student
Nakayama, Yuki	2012	2014	TCSVM student
Patellos, Katherine	2012	2015	TCSVM student
Picciotto, Emily	2012	2015	TCSVM student
Reiss, Eliza	2012	2014	TCSVM student

Sharp, Sarah	2012	2015	TCSVM student
Stephan, Sarah	2012	2014	TCSVM student
Sweeney, Joseph	2012	2015	TCSVM student
Topliff, Elizabeth	2012	2014	TCSVM student
Tuttle, Emerson T.	2012	2014	TCSVM student

Key Research Accomplishments

The goal of this program was to provide research training to veterinary students by allowing them to participate either in a research project over the summer months (Summer Research Program from June to August) or a year-long hypothesis driven research (DVM/MS-CBS). The short term summer research training usually does not always result in the publication of a manuscript, as the trainees are still learning the research methods and the project is usually not completed during the summer months. Thus, the focus was to get the trainees interested in research including how to evaluate and prepare scientific publications. The major findings are summarized below.

- Construction of global positioning system map to describe the distribution of bovine rabbies.
- Immunohistochemical testing for rabbies
- PCR technique to detect bat and viral DNA
- Seroprevalence of antibodies to Australian bat lyssavirus
- Rose Bengal agglutination test to detect seroprevalence of Brucellosis melitensis
- Participatory disease surveillance method for the detection of highly pathogenic avian influenza
- Development of multiplex PCR technique for differentiating among three species of mycobacterium.
- Comparison of tuberculin test and nested PCR to detect bovine tuberculosis
- Streptomycin, ampicillin and tetracycline resistant isolates were the most prevalent type of resistance phenotypes in both gull and wastewater isolates.
- Chronic infusion of allopregnanolone into the medial preoptic area (MPOA) appears to increase anxiety.
- A greater percentage of juvenile samples were found positive for parasites than adults monkeys in the rainforests of Costa Rica.
- Initial results suggest presence of chronic aflatoxicosis in Indonesian domestic ducks.
- The effect of reproductive experience on anxiety-like behavior may be due in part to changes in the modulation of anxiety-like behavior by activation of the estrogen receptor α subtype in primiparous females.
- 85% of dogs diagnosed with sarcoptic mange showed noticeable improvement in clinical signs when treated with promeris.
- An important perception revealed by this ethnographic study is that many poultry owners in West Java believe they can recognize HPAI, even though the signs they describe as pathopneumonic for HPAI are seen with endemic Newcastle disease and other diseases.
- Saliva has the potential to be a diagnostic sample for hormonal analysis and for pathogenic bacteria and viruses in free-living mountain gorillas.
- A cross-sectional cluster sample survey showed that the dog sterilization and vaccination program had a positive impact on rabies control program in Rampur, Nepal.
- Rabies seroprevalence of Mexican bats revealed a statistically significant difference between bats in disturbed (60.0%) and undisturbed (27.9%) locations. The disparity in seroprevalence may be due to differences in diversity and abundance of bat species at the different locations.
- A cross-sectional, descriptive study in Indonesia revealed that Muscovy ducks are raised in backyard flocks, frequently with chickens and ducks, but also with other species of birds and cattle. Because the highly pathogenic avian influenza (HPAI) virus can be spread via fomites, implementation of proper hygiene should decrease the prevalence of HPAI in the environment.
- Health status of tortoises is not significantly affected by translocation to another habitat used to protect gopher tortoise.

- A comprehensive set of health-based variables for the Sharpnose and Bonnethead sharks in the southeast Atlantic was established
- The results of a participatory-based questionnaire showed that access to government-sponsored veterinary service was higher outside than inside the Limpopo National Park (LNP) in Gaza province, Mozambique. This raises the potential for increased disease transmission as high numbers of livestock inside the LNP continue to be exposed to a reservoir of vectors for tick born disease.
- Transmission of tuberculosis may occur among elephants at different stables.
- The combination of Newcastle Disease (ND) control and improved husbandry practices shows promise as a means to improve the nutrition and overall quality of life of the people living in in Gaza Province, Mozambique.
- It is important to maintain recommended parameters for the cold chain from Dar Es Salaam to Singida for the storage refrigerators without the loss of activity of the thermotolerant I-2 Newcastle disease vaccine.
- It is possible to measure rotational and translational motion of equine meta-carpophalangeal joint in 3D space with a high degree of accuracy (± 0.08 mm) using the XROMM (X-ray Reconstruction of Moving Morphology) model.
- Males and females nesting at low and high densities play an equal role in the dissemination of antibiotic resistance.
- *T. gondii* seropositive cats had significantly lower levels of hemoglobin, red blood cell counts, and hematocrit ($P < 0.05$) than seronegative cats.
- Smoking or cooking of bush meat can be incomplete, and does not completely destroy the genomic integrity of some viruses. This result raises concerns about the potential risk of infection to either animals or people exposed to imported bush meat.
- The PCR screen was a quick and effective means of screening for avian haemoparasite.
- N-terminal pro-CNP was a useful diagnostic biomarker for septic peritonitis in dogs.
- The body condition scores of captive elephants declined after the age of 40 years suggesting that the nutritional needs of older elephants is different from younger elephants.
- There is a lack of knowledge about general poultry husbandry and poultry disease in Zambians.
- Pulse oximetry can be a valuable tool for estimating arterial oxygen status in the Asian elephants.
- Equirab (Bharat Serums and Vaccines Limited, Mumbai), the equine rabies immunoglobulin (RIG) currently used at Sukraraj Tropical and Infectious Disease Hospital in Nepal, is a safe alternative to human RIG for rabies exposure prophylaxis.
- The scFvs of Shiga toxin Stx2 A subunit-specific HuMAb 5C12 and Stx2 B subunit-specific HuMAb 5H8 have been cloned and expressed in the expression *E. coli* host.
- Ducks can be used to control snails and thereby help control *A. cantonensis* infection in gibbons in captivity.
- An HPLC-MS assay was developed to measure tissue terbinafine (used to treat white nose syndrome in bats) concentrations of less than 0.1 μg /tissue sample.

Reportable Outcomes (2007-2012):

1. Twenty six (26) trainees participated or are participating in the DVM/MPH joint degree program
2. Twenty one trainees (21) participated or are participating in the DVM/MS-LAM joint degree program
3. Ten (10) trainees participated or are participating in the DVM/MS-CBS joint degree program
4. Eighty three (83) trainees participated in the summer research program
5. Four trainees joined US Army Veterinary Corps.
6. Nine trainees are engaged in biomedical research.
7. Seven trainees received further training in laboratory animal medicine at other institutions.
8. Five trainees are enrolled in PhD program.
9. Two trainees have joined government agencies.
10. Two trainees received Fogarty Clinical Fellowship award.
11. One trainee received national Merit Veterinary Scholars award in 2013

Conclusion:

This multi-year program to increase veterinary research manpower is considered to be successful, as evidenced by the number of trainees recruited in the program and the activities of the graduates. All trainees in the combined degree program are making satisfactory progress. Although only few peer-reviewed publications have resulted from the research conducted by the trainees, some of the trainees presented their work at national meetings (see below). No major problem was encountered with recruiting or maintaining trainee interests in the training program.

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Appendix 1: DVM/MS-CBS thesis summary

Appendix 1

DVM/MS-CBS thesis summary

Name of the Trainee: Karyn Elizabeth Von Iderstein

Mentor: Andrew M. Hoffman

Thesis title: The role of stem cell antigen (Sca-1) expression in the control of lung regeneration and progenitor cell function.

Summary: Stem cell antigen-1 (Sca-1), a murine-specific stem cell marker, plays a role in the regulation of proliferation and differentiation of progenitor cells during tissue repair. Sca-1 has been used as a marker to isolate pulmonary stem cells, including the bronchioalveolar stem cell (BASC), which displays progenitor cell properties in vitro and increases in abundance during lung regeneration in vivo. To determine the role of Sca-1 during lung regeneration, we performed pneumonectomy (PNY) in 1) C57Bl/6 mice, which express high levels of Sca-1 in bone marrow, 2) BALB/c mice, which have markedly lower Sca-1 expression in bone marrow, and 3) Sca-1^{+EGFP} reporter mice, which are heterozygous for Sca-1 and express enhanced green fluorescent protein (EGFP) targeted to the Sca-1 locus. Lung function, BASC abundance, and Sca-1 expression were analyzed in each strain following PNY. Our studies showed that while C57Bl/6, BALB/c, and Sca-1^{+EGFP} mice regrew their lungs following PNY, there were differences in this process between strains. Sca-1 (or GFP) expression increased following PNY and both BALB/c and Sca-1^{+EGFP} strains show a decrease in BASC abundance relative to C57Bl/6. These results provide justification for further studies to determine the importance of Sca-1 (using a null model) as well as BASC in lung regeneration.

Name of the Trainee: Elliot Garber

Mentor: Saul Tzipori

Thesis title: Molecular characterization of *Giardia* and *Cryptosporidium* isolates from children and cattle in southern India.

Summary: *Giardia duodenalis* and *Cryptosporidium* spp. are leading causes of diarrhea in India, but little is known about the assemblage types or species commonly infecting children and calves from rural areas. Zoonotic transmission has been hypothesized to be a significant factor in human giardiasis and cryptosporidiosis worldwide, but the relative importance of zoonotic and anthroponotic transmission is unknown. Recent molecular studies identify the genetic heterogeneity present among *G. duodenalis* and *Cryptosporidium* isolates and indicate that further genetic characterization is necessary to more fully understand the population structures of these parasites and design methods for their control. Stool samples from 70 children and 56

calves of the same households were collected over a three-month period in a rural district of southern India. PCR-RFLP at the triose phosphate isomerase (*tpi*) gene and at the small subunit ribosomal RNA (*ssu*-rRNA) locus were used to detect and identify *G. duodenalis* assemblage and *Cryptosporidium* species, respectively. *G. duodenalis* assemblages A and B were isolated from children and cattle, and assemblage B was the predominant type identified in both. Five households were identified in which children and calves were both infected with *G. duodenalis* assemblage B during the study period, suggesting the possible involvement of zoonotic or anthroponotic transmission cycles. Microscopy and PCR-RFLP results for *Cryptosporidium* showed low prevalence of *C. parvum* infection in both children and calves. These results provide important insight into the population structure of *G. duodenalis* and *Cryptosporidium* in rural southern India and suggest the possibility of zoonotic transmission of *G. duodenalis* assemblage B.

Name of the Trainee: Marieke H. Rosenbaum

Mentor: Andrew M. Hoffman

Thesis title: Foil or Function? Resident phagocytes interfere with engraftment of locally-derived mesenchymal cells in the murine lung.

Summary: Lung diseases in humans and animals are characterized by damage of the normal pulmonary architecture and by loss of important precursor cells. Autologous locallydelivered cell-based therapy is a new method to approach the repair and restitution of lung cell populations, but the exact cells to be employed, and

their fate after intratracheal injection have yet to be determined. In this study we isolate a potentially therapeutic contractile interstitial mesenchymal cell based on bright expression of the $\beta 1$ integrin. We describe the *in vivo* and *in vitro* characteristics of this cell and demonstrate that superior retention and incorporation of PKH26-labeled cells that expressed the integrin $\beta 1$ at very high levels at the time of injection are improved in a regenerating lung model and are partially dependant on interactions with pulmonary dendritic cells and/or macrophages. Furthermore, by fluorescence activated cell sorting (FACS) and immunofluorescence (IFA), we demonstrate that the great majority of retained PKH+ cells 14 days following cell injection express dendritic cell and macrophage markers CD45.2 and CD11c and that PKH+ dendritic cells appear to be immature based on activation markers CD40 and CD80. Incorporation of PKH+ cells into surrounding alveolar tissue is apparent based on examination of frozen sections, suggesting therapeutic function. However, PKH+ cells do not express epithelial markers characteristic of fibroblasts or type 1 or 2 pneumocytes and do not express matrix proteins collagen type IV and laminin, thus foiling perceived engraftment. Our results highlight the need to further examine the mechanisms behind interactions of locally delivered autologous cells with resident immune cells in all areas of cell-based therapy and to determine if this interaction holds therapeutic potential in the lung.

Name of the Trainee: Jessica Perry Hekman

Mentor: Alicia Z. Karas

Thesis title: Identification and Reduction of Stress in Hospitalized Dogs

Summary: Psychogenic stress has been shown to affect medical outcomes in humans and animals. It is likely that hospitalized dogs experience psychogenic stress as a result of their hospitalization, and that this stress affects their health outcomes. However, little is known about the specific components of hospitalization stress or about its effects on health outcomes. Additionally, no validated tools exist to diagnose high stress in canine patients, nor are stress-reducing interventions widely accepted. Therefore, improved tools for evaluation of stress in hospitalized dogs would be useful in studies on the effects of stress on that population, and clinically to evaluate canine patients in need of intervention. A population of hospitalized dogs was video recorded, for behavior analysis, and salivary cortisol levels were taken. Associations were found between salivary cortisol level and three behaviors, “resting the head on the paws or ground,” “not panting,” and “lip licking.” A research scale and a clinical scale for stress evaluation were developed based on these four behaviors. The behaviors and scales were tested in a second population of dogs. One behavior, “lip licking,” did not have useful associations with salivary cortisol level in this new population. Additionally, the scales were tested to determine minimum length of observation. Two minutes of observation was found to be insufficient, but twenty minutes of observation to be sufficient, to predict salivary cortisol levels using the research scale. The research scale was revised to use only the behaviors having useful associations with salivary cortisol. Finally, the efficacy of Bowen, a non-pharmacologic method of stress reduction believed to be effective in dogs but not previously studied, was tested by comparing pre- and post-intervention behaviors, stress evaluation scores, and salivary cortisol levels in hospitalized dogs, using both active and negative controls. Bowen reduced salivary cortisol levels post-intervention in hospitalized dogs, but this difference was not significant.

Name of the Trainee: Katherine Jane Megquier

Mentor: Kerstin Lindblad-Toh

Thesis title: Clinical and Genomic Characterization of Canine Hemangiosarcoma

Summary: Hemangiosarcoma (HSA) is an aggressive cancer associated with short survival times despite treatment. The Golden Retriever has a high risk of HSA, suggesting that there are genetic risk factors involved. This thesis is divided into three parts: a clinical variables study, a genome-wide association study, and a gene expression study.

We examined a retrospective case series consisting of 131 Golden Retrievers with HSA to look for factors associated with survival time and age of onset. Our results showed that males castrated at ≤ 2 years had a significantly lower age of onset (median 8.40 years, IQR = 7.41 – 9.82, $n = 8$) than males castrated at > 2 years (median age: 10.84 years, IQR = 10.00 – 11.80, $n = 8$) ($p = 0.009$). This did not hold true in females. This finding could give insight into possible hormonal mechanisms of HSA and suggests that delaying castration may be an effective way to delay onset of this cancer in male Golden Retrievers.

A genome-wide association study was performed with Golden Retrievers using DNA from 142 HSA cases and 194 controls. Samples were run on Illumina CanineHD Genotyping BeadChips. The four top associated regions were found on: chromosome 5 at ~32.9 megabases (Mb) ($p = 1.8E-07$, EMP2 = 0.022), chromosome 11 at ~40.8 Mb ($p = 1.8E-06$, EMP2 = 0.152), and on chromosome 27 at ~37.4 Mb ($p = 3.3E-06$, EMP2 = 0.245) and ~40.8 Mb ($p = 1.6E-06$, EMP2 = 0.139). There are interesting candidate genes in these regions that will be explored further once fine-mapping and resequencing are completed.

In the gene expression study, we isolated total RNA from HSA tumor and normal tissue samples from multiple breeds. The samples were run on Affymetrix Canine 2.0 Genechips. We compared 23 HSA tumor samples to 18 normal tissue samples. There were 1898 differentially expressed probes with an adjusted p-value of less than 0.05 and a combined fold change greater than 2. Many of these genes have been shown to play important roles in other cancers, particularly in metastasis, invasion, and in resistance to apoptosis, and could potentially be targeted in new hemangiosarcoma therapies.

Name of the Trainee: Jana E. Mazor-Thomas

Mentor: Florina Tseng

Thesis title: Bench and Behavior: Two Non-invasive Methods for Investigating Pain and Analgesia in Red-tailed Hawks (*Buteo jamaicensis*).

Summary: Pain management, though a vital component of humane care and a significant contributor to health and recovery, is poorly understood in wild birds. Considerable variation in response to different therapeutic protocols is likely to exist between species, ages, and sexes, complicating efforts towards effective clinical practice. In addition, traditional methods of analgesimetry, developed for laboratory use, are potentially physiologically inappropriate for wild raptors, and there are ethical concerns related to inflicting pain on captive wildlife. It is also unclear how well results from these tests actually reflect pain or analgesia in an injured animal. In this study, two broader avenues of investigation were pursued: mapping of opioid receptors in a novel clade (raptors), in combination with a behavioral analytical model that has shown promise in other species but which has not yet been used in wild birds. Our data indicate that trauma is associated with significant reductions in several measures of voluntary activity, and that this behavioral alteration is not likely to be the result of buprenorphine administration during hospitalization. A separate study on feeding behavior indicates that birds with orthopedic trauma are slower to resume voluntary feeding after admission to the hospital, suggesting that changes in feeding behavior may also be a useful marker of pain status. Immunohistochemical mapping of mu and kappa opioid receptors has been partially completed and identification of relevant neuroanatomic structures is ongoing.

Name of the Trainee: Bronwen A. Childs

Mentor: Karl Kirker-Head

Thesis title: 3-D Motion Analysis of the Equine Fetlock Joint: A Novel Imaging Modality and its Application in the Evaluation of Protective Leg-wear

Summary: The objective of this study was to validate the use of flexible electrogoniometry (EGM), an affordable and user-friendly modality, for the purpose of characterizing the effects of metabolic fatigue and protective legwear on equine metacarpophalangeal joint (MCPJ) kinematics. The specific hypotheses of the study were: *H1*) using an equine cadaver limb model, the EGM can be applied in a manner that will reflect actual MCPJ flexion and extension angles with accuracy (± 2 degrees); *H2*) absolute maximum extension angles, angular velocity, stride duration, and stride frequency calculated from EGM-derived data recorded *in vivo*, will fall within the range of values (\pm one standard deviation) previously reported for non-fatigued horses; *H3*) hyperextension of the MCPJ is exacerbated by metabolic fatigue; and *H4*) any hyperextension and increased angular velocity of the MCPJ encountered in the fatigued horse can be effectively attenuated using novel protective legwear.

The thesis comprised 4 study phases. The first phase uses an equine cadaver forelimb to determine an optimal application technique for the EGM hardware on the limb and to evaluate the selected technique's accuracy (H1). The next phases were completed as 3 *in vivo* treadmill assessments, which used a single gelding (540±10kgs, 164.6cm high at the top of the withers). The first *in vivo* phase analyzed the reliability of the preferred application technique for the EGMs, derived from the cadaver limb phase above, when applied to the live horse. It also evaluated how well our data agreed with existing scientific literature (H2). The second *in vivo* phase attempted to characterize the effects of fatigue on MCPJ kinematics by comparing data from the non-fatigued and fatigued horse (H3). The final portion of this study evaluated the effects of prototype legwear on MCPJ kinematics (H4).

Our data reflect an accuracy of ±2.16 degrees for our preferred *in vitro* technique of EGM fixation, which is in general agreement with the accuracy reported by the EGM manufacturer (±2 degrees) (H1). Additionally, the kinematic and temporal variables reported in this study were consistent with previously published data (H2). Therefore, we can accept our first (H1) and second (H2) hypotheses. However, the non-fatigue and fatigue data obtained were inconclusive, so we rejected our third hypothesis (H3). Finally, while unable to test the protective legwear in the fatigued animal, in the non-fatigued horse we demonstrated its ability to influence normal MCPJ kinematics and the temporal variables of the horse's stride (H4).

Collectively, these data imply the inherent value of electrogoniometry as a valid means of characterizing MCPJ kinematics in the horse. The preliminary kinematic and temporal data we derived are consistent with data generated using other methodologies. The influence of fatigue on MCPJ kinematics remains to be characterized but the potential for protective legwear to limit normal joint kinematics in the non-fatigued horse was demonstrated.

Name of the Trainee: Marian Elise Schenk

Mentor: Raimon Duran-Struuck

Thesis title: *In Vivo* Development of Aggressive Swine B-cell Lymphoma in NSG Mice for the Creation of a Large Animal Tumor Model

Summary: Swine have been used extensively in biomedical research as a pre-clinical large animal model. For the past 40 years, the Massachusetts General Hospital (MGH) major histocompatibility complex (MHC) defined miniature swine have been utilized for transplantation and cancer studies. The goal of this study was to develop aggressive swine tumor cell clones for serial transfer in MHC matched swine. Two tumor cell lines isolated from swine were studied. One was a swine B-cell lymphoma subline LCL13271 that originated from a transplanted animal that developed post-transplant lympho-proliferative disease (PTLD). The second was a spontaneous chronic myeloid leukemia (CML14736). In order to select for aggressive tumor variants, these lines were injected into NOD/SCID IL-2 receptor γ -/- (NSG) mice. Tumor induced mortality in mice injected with CML14736 was 68% while 100% of mice injected with LCL13271 succumbed to PTLD. The PTLD cell line was selected for further development based on its aggressive traits and infiltration of the kidneys, liver and spleen of all animals. Re-injection of cells into NSG mice showed an increase in tumor burden and increased metastasis. An attempt to induce PTLD using the original tumor in an MHC matched swine failed. Tumor cells transduced with green fluorescent protein (GFP) to facilitate tumor tracking, demonstrated a decrease in survival time by 17 days ($p=0.004$) compared to GFP- cells. GFP tumors were successfully tracked for 48 hours in circulation when injected into a second swine. In summary, we report the development of an aggressive GFP+ PTLD swine tumor in NSG mice with the potential to be utilized in large animal tumorigenesis studies.

Name of the Trainee: Kristy L. Meadows

Mentor: Elizabeth Byrnes

Thesis title: Effects of aging, gender, and reproductive experience on anxiety-like behavior and regulation of the relaxin family peptide system

Summary: Anxiety disorders affect approximately 40 million Americans, with women nearly twice as likely to be affected as men. Hypothalamo-pituitary-adrenal (HPA) axis dysfunction has been associated with anxiety

disorders in humans and rodent models. The HPA axis is a neuroendocrine regulatory system that controls the response to stress and anxiety. Corticotropin releasing hormone (CRH) produced in the paraventricular nucleus (PVN) of the hypothalamus and in the amygdala (AMYG) plays a key role in activation of the HPA axis and behavioral activation in response to stress. Furthermore, a newly discovered neuropeptide produced in the brainstem, relaxin 3 (RLX3), has been hypothesized to play some role in the response of the HPA axis and behavioral activation. Studies show that male and female subjects show noteworthy differences in the activation of the HPA axis in response to fear or anxiety. Similar to the sex differences described in HPA axis responsiveness, age-related change is also routinely reported. In addition, recent studies have shown that reproductive experience may result in changes in the male brain in response to stress and it is well known that pregnancy results in changes in the female brain. In the current study, we examined potential sex and age differences as well as differences in the effects of reproductive experience on anxiety-like behavior, HPA axis activation, and response of the relaxin family peptide system through exposure to a mild stressor. We also examined potential sex differences in the effects of the end product of HPA axis activation, glucocorticoids, on production of RLX3. In addition, we examined the effect of central administration of RLX3 on anxiety-like and motivational behaviors. The results of the present set of studies demonstrate that both age and reproductive experience modulate anxiety-like behavior and activation of the HPA axis. The nature of these effects appears qualitatively similar in both males and females. However, changes in the relaxin family peptide system differ between the sexes, suggesting that control of this particular system may be sex-specific. Additionally, the results suggest that RLX3 itself may be capable of modulating anxiety-like behavior in males.

(Last Page)